Chapter

Microbiological Indices for Diagnosis of Heavy Metal Contaminated Soils

Sukirtee Chejara, Paras Kamboj, Y.V. Singh and Vikas Tandon

Abstract

Heavy metal contamination has gained popularity worldwide due to their persistent nature in the environment, on the top of that non-biodegradable nature makes its accumulation easy to toxic levels. Understanding the nature of contamination has become a major concern before heavy metals deteriorate the quality of soil; to diagnose heavy metal pollution suitable indices are required. Microbial indices gaining importance because of their sensitive nature towards change in surrounding, which is the imperative quality required to select microbes as environmental indicators. Albeit enough literature is present related to this topic but the information is scattered so role of this chapter is imperative. The chapter will be helpful for the reader to provide a thorough understanding of merits and demerits of microbiological indices for heavy metal contaminated and restituted soils. The changes in microbiological indices and their mechanism of response towards heavy metal stress are effectively summarized. Research gap and future needs of microbial diagnosis of heavy metal contaminated soils are discussed.

Keywords: heavy metals, soil contamination, microbial indices, soil pollution, soil health

1. Introduction

Heavy metals are very crucial for maintaining the life cycle of living organisms. Albeit they are important but excessive accumulation of heavy metals is harmful for environment as well as for human health. Excessive accumulation of heavy metals in the soil may take different pathways, which may be through industrial residue, chemical manufacturing, pesticides and fertilizers, sewage irrigation, metal plating etc. but all sources are principally result of anthropogenic activities [1]. Heavy metal pollution in soils is present in different parts of the world including Spain, United states, France and India are in worst condition by Cd- contaminated soils [2]. Urban soils of Naples city and Mexico city is contaminated with Cu, Pb and Zn [3, 4]. Due to non- biodegradable nature of heavy metals their persistence in nature is very long which harms soil ecological environment [5]. Earlier studies proved that high heavy metal concentration cause certain harmful effects on microorganism as dysfunction of cell, protein degeneration, and sometimes destruction of plasma membrane [6]. Above that heavy metal contamination affects enzyme activity of microorganism, DNA sequencing as well as abundance is also affected

by heavy metal contamination. Thus, it is highly important to choose such indices which are accurate and efficient for the diagnosis and analysis of quality of heavy metal contaminated soils, so that preventive measures chosen beforehand and further deterioration of soil quality can be controlled as well as suitable remediation technique could be employed on time. Soil quality can be diagnose using sensitive microbial indices, which are monitoring of soil microbial changes before and after contamination or some remnant part of land under observation. In a general perspective soil having higher microbial population or activity performs better and can be called as good quality soil. Using microbes for diagnosis have several advantages *i.e.* (1) Microbes are active participants of soil ecosystem [7] highly sensitive for heavy metal contamination than plants and animals growing in the similar conditions [8]; (2) microorganisms are closely related to pollutant degradation and soil fertility conditions [9]; (3) microbial analysis requires a very small amount of sample, quick to perform, simple and cheaper [10]. One should always include some ecologically relevant attributes while diagnosing soil quality so that they give better performance while studying ecosystem quality. Microbiological indicators mainly include study of microbial population, microbial diversity, function and activity. If the indices are correctly selected they will give better information about heavy metal polluted soils. Albeit information about microbial indices are available in literature but that information is scattered. This chapter provides information about merits and demerits of using microbial indices for heavy metal contaminated soils. The changes occurred in different indices and their possible mechanism under heavy metal stress were studied comprehensively and summarized.

2. Diagnosis based on microbial abundance

Abundance of functional gene is a genetic diagnosis method of understanding heavy metal contaminated soils. Presently genes related to nitrogen transformation are gaining popularity in diagnosis of target soil. In the process of nitrification ammonium ($NH_4^+ - N$) is converted to Nitrite (NO_2^-) and ultimately to Nitrate (NO_3^-) [11]. In the nitrification process ammonia oxidation is the rate limiting step in the global N-cycle [12, 13]. Ammonia oxidation is carried out by Ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB) [14]. They contains different enzymes to carry forward the process like AMO, HAO and NXR. AMO protein contains alpha, beta and gamma subunits as it is a trimeric membrane-binding protein, units alpha, beta and gamma is encoded by genes amoA, amoB and amoC genes respectively [15]. Nitrite oxidation is carry forward by a group of microbes i.e. nitrite oxidizing bacteria (NOB) [16]. Heavy metal contamination is widely Diagnose using ammonia oxidizing gene as markers mostly amoA gene due to its conservative coding. When abundance of amoA gene is compared for AOB and AOA in a Cu contaminated soil it is found that amoA gene has a negative correlation with Cu concentration [17]. When the sensitivity is compared AOB amoA gene was found more sensitive than AOA amoA gene. AOB and AOA amoA gene abundance is reduced when the soil is contaminated with As and Pb, the sensitivity of AOB was found higher than AOA [18]. Similar results were found in case of sensitivity when studied a Cd contaminated soil [11]. AOA found less sensitive than AOB it may be because of AOA have metal reducing ability and heavy metals are generally less toxic when they are in their reduced state i.e. lower valance state [19] which ultimately is beneficial in metal detoxification. AOA have more rigid cell membrane than AOB.

Just opposite to the above recorded observation, scientist indicated that in a Zn contaminated soil abundance of AOA amoA gene decreased quickly than ABO [20].

In long term Zn tolerance development AOB amoA gene copy and transcript enhanced hence AOB community structure also changed, And AOA failed to respond towards Zn [21, 22]. Albeit the abundance of amoA gene of AOA was dominated in second year but expression from the genes were not detected [20]. Response of AOA community is not that clear till now with the available literature further details are needed to understand whether AOA can adapt to long term contamination. AOA may use other processes to fulfill their energy requirement or they may survive in their dormant state. Despite cultivated AOA clusters are few in numbers, so response of AOA to external environment is so far needed exploration. Remediation of contaminated soil exhibit changes in amount of ammonia oxidation genes. Application of biochar and alfalfa enhanced abundance of amoA gene of AOA and AOB in a heavy metal and fungicide contaminated soil [11]. Abundance of AOB amoA gene increased with application of biochar in a Cu and Pb contaminated soil when the soil is remediate using biochar and compost [23].

However some scientist reported gene copy number is a weak indicator for heavy metal pollution. There was no significant change in gene abundance of AOB or AOA amoA gene when a soil is treated with Hg [24]. This may be because of Hg tolerant ammonia oxidizing community present in soil from before or may be application of Hg may induce tolerance in the community [24] thus from this study it is found that amoA gene did not respond towards heavy metal pollution, but for its confirmation we need further exploration of the nature of gene. Gene transcript number is found a better index than gene abundance when talking about indices of soil quality. In a study it is found that there was a decrease in amoA gene transcript number of AOB and AOA by three and four order of magnitude, while gene copy number remained unchanged in a one week Zn treatment [21]. Hence from the above discussion it can be concluded that heavy metal pollution cannot be predicted accurately on the basis of change in gene abundance of AOA and AOB further research is still needed in this aspect. Furthermore we cannot judge the change in any one of AOA or AOB separately there may be some sort of interaction among both the community while dealing with heavy metal toxicity [19]. So it is recommended to monitor the change in both the community simultaneously other than thinking separately. Sometimes increase in growth of microbial community may be a response of toxic effect [25]. Till now only AMO genes are explored to some extent while HAO gene and NXR gene did not received much attention it may possible they may express well as a diagnosing tool than AMO in heavy metal contaminated soil.

3. Response of denitrification genes

During denitrification nitrate is converted to dinitrogen through several intermediate products $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ [26, 27] different reductase enzymes are involved at different stages of intermediate product for nitrate reduction nitrate reductase (Nar), for nitrite reduction nitrite reductase (Nir), for nitric oxide reduction nitric oxide reductase (Nor) and for nitrous oxide reduction and nitrous oxide reductase (Nos). Nitrate reductases (Nar) encoding is done by operons of nas, nar and nap. Encoding of nitrite reductase (Nir) is done by nirK and nirS, while nitric oxide reductase (nor) consist of norB and norC. Nitrous oxide reductase (nos) contains nosZ, nosR and nosD etc.

Denitrifying enzymes encoding genes are very sensitive towards heavy metal stress, they characterize denitrifier community and helpful in diagnosing soil quality. Different studies have been carried out to study the relation of denitrifying enzymes and heavy metal stress and found that reduction in genetic diversity is the most common inhibitory effect of heavy metal stress [28, 29]. Research conducted

on Pb contaminated soils and found that nirK gene community diversity was reduced due to Pb contamination [30]. Enzyme involved in different reduction steps showed significant difference in Cu tolerance in a study conducted it was reported that diversity of nirS, nirK and nosZ genes decreased with the increase in Cu concentration [31]. Increase in Ag concentration lead to decrease in nirK gene copy number but diversity of nirK gene increased [29]. Under Hg stress nirS gene diversity increased. Different denitrification genes respond differently towards same environmental pressure [32, 33] thus, selection of sensitive indicators becomes mandatory for detection of soil pollution.

Abundance of nirS gene changed significantly under Hg stress while no change in nirZ gene was observed under all given treatments, this proves nirS gene more sensitive than nirZ gene [34], while it can be said that nosZ denitrifier is resistant under different pollution condition in soil and shows more stability [21, 27]. Effect of different remediation strategy were observed. Abundance of denitrification genes (narG, nirK, nirS) except nosZ increased with application of alfalfa and biochar in a heavy metal and fungicide contaminated soil [11]. Denitrifying genes shows different patterns while diagnosing heavy metal pollution, hence further research is needed for better information base. However nosZ gene found less sensitive to heavy metal pollution than denitrifying genes, further its resistance need to be study.

4. Microbial biomass

Microbial biomass in soil include living microorganism present in soil i.e. fungi, bacteria, algae and protozoa [35]. Microorganisms contain usually carbon, nitrogen, phosphorus and sulfur but mainly their population is expressed as microbial biomass carbon. Terrestrial ecosystem organic matter dynamics is affected by microbial biomass being an important component of organic matter in soil [36]. Microbial biomass have a direct correlation with soil condition [36], there are sufficient evidences which proves the sensitivity of microbial biomass with increase of heavy metal stress [37–39]. Microbial biomass can be used to predict soil quality. Higher microbial biomass in soil indicates good functional quality and will be able to store more nutrients and regulated nutrient cycles [40]. Heavy metal stress severely inhibits microbial biomass [8, 40]. Increase in Cd concentration leads to decrease in C_{mic} in soil [41]. A negative correlation between soil microbial carbon and heavy metal concentration (Cd, Pb) is indicated [42].

Under heavy metal stress microbes requires more energy for their survival which required more consumption of substrate, resulting less substrate left for other microbes. This limits their growth [5, 39]. Albeit there will be declined microbial biomass but it does not indicate population extinction, more resistant species will fill the gap with their presence, microbial ecosystem will remain enriched [43]. On the other hand remediation strategy helps in increasing microbial biomass, which indicated improved soil condition. Soil replacement found to be helpful in increasing carbon when metal concentration decreased in soil (Cd, Cu, Pb, and Zn) [36]. C_{mic} may not respond sometimes effectively to stimulation of heavy metal, any correlation between microbial biomass and heavy metal was not found [39, 43]. [44] found that there were no significant relation between C_{mic} and soil- soluble Cu. No correlation found between heavy metals (Cr, Cd, Pb, Zn, Cu) and carbon [45]. Microbial biomass Nitrogen (N_{mic}) also serve a good indices for soil quality assessment, it is found associated with heavy metal content in different cases [46]. N_{mic} decreased with increased heavy metal content [43]. While inconsistent and weak downward trend of N_{mic} under metal contaminated sites (Cd, Cu, Pb, Zn)

was observed [37]. N_{mic} found less correlated with heavy metal pollution than $C_{mic.}$ Nitrogen at severely polluted areas of metal contamination was 64.4% of non-polluted area while C_{mic} accounted only 31.6% [40]. Albeit individual microbial biomass is highly sensitive towards change in soil condition but it has certain limitation while serving as indices for soil monitoring. One cannot predict change in microbial structure only through microbial biomass observation. Short term response of microbes to heavy metal contamination does not predict soil quality in long run, even if the soil environment is same. At lower metal concentration (Cd/Cu/Zn) C_{mic} changed in long long-term experiment while no change were observed in short laboratory test [47]. Microbial biomass is highly dependent on soil physical, chemical and biological properties, which are helpful to blur the toxicity of heavy metals. Soils with more labile carbon increases C_{org} in soils [48]. Soil particle size also affects toxicity of heavy metals, heavy metal toxicity (Pb, Cd, Zn and Cu) to C_{mic} was more prevalent in coarse fraction of soil than clay fraction [48]. Different biomass related ratios to heavy metals also have been explored. C_{mic}/N_{mic} ratio is helpful in controlling microbiologically operated nutrient cycling and availability [49], this ratio is an important indicator of soil condition. There are sufficient studies available which indicate that heavy metal stress can induce change in C/N ratio [46, 49]. Under heavy metal stress C_{mic}/N_{mic} may increase due to increase in tolerant fungal component. Huge difference between C/N ratio of bacteria and fungi support this increased ratio with increase in fungal population, C/N ratio of bacterial species 3.5:1 while for fungal species this ratio ranges from 10:1 to 15:1 [46].

Soil microbial biomass	Contaminants	Remediation	Results	References
C_{mic}	Cd	Plantation of *Eulaliopsis binata*	Negative correlation	[41, 42, 47]
	Cd, Pb	Plantation of *Sedum plumbizincicola*	Negative correlation, Metal content decreases, C_{mic} increases	[42, 44, 59]
	Cd, Cu, Pb, Zn	Soil replacements in trenches+ planting Eucalyptus in contaminated soil + Natural vegetation	Metal content decreases, C_{mic} increases	[60]
		Soil replacements in trenches+ planting Eucalyptus in contaminated soil along with uncontaminated soil in upper 20 cm soil layer+ *Brachiaria decumbens*	Metal content decreases, C_{mic} increases, C_{mic} was found 100% higher than earlier method	[36]
N_{mic}	Cd, Cr, Cu, Pb, Zn	Cultivation of Eucalyptus binata	No correlation	[45]
	Cd, Pb	Cultivation of Eucalyptus binata	Metal content decreases, N_{mic} increases	[59]
	Cu, Zn, Cd, Pb, Ni, Mn	Cultivation of Eucalyptus binata	Polluted sites, decreased N_{mic}	[40]
	Cd, Cu, Pb, Zn	Cultivation of Eucalyptus binata	Polluted sites, decreased N_{mic}	[37]

Table 1.
Heavy metal pollution with relation to microbial biomass.

Fungal species appear more resistant to heavy metal pollution than actinomycetes and bacterial species [50, 51]. Fungal/bacterial population ratio is considered as a good soil health indicator [48, 52]. Bacteria and fungi play dominant role in nutrient availability and organic matter dynamics being the major population governing soil microbial biomass i.e. about 90% of total microbial biomass [48]. Heavy metal stress cause bacterial mortality which enhances carbon release, this carbon is used by resistant fungal population for their growth [25]. However this index is not generally used for diagnosis of soil pollution. C_{mic}/C_{org} is also a good indicator of soil heavy metal pollution. Different studies indicate under heavy metal pollution C_{mic}/C_{org} ratio decreases [53, 54]. In a study C_{mic}/C_{org} ratio is found negatively correlated with As and Cu contamination [55, 56]. While in a study it was observed that C_{mic}/C_{org} ratio increased with decrease in heavy metal stress (Cu, Zn) [48]. When C_{org} is used by microorganisms for their respiratory metabolism the efficiency of conversion of C_{org} to C_{mic} reduced hence ratio of C_{mic}/C_{org} also declines [55, 57]. Few scientist claim that the ratio of C_{mic}/C_{org} is significant in non-contaminated soils, but for metal contaminated soils this relation even may not exist [58]. Not Any change in C_{mic}/C_{org} nor any obvious trend was present under heavy metal contaminated soil (Zn, Cd, Pb, Cu) [46]. Hence in microbial biomass or in related ratios no consistent and clear change is observed with heavy metal pollution. This ratio does not reveal any change in population structure. Hence none of them is suitable solely as an indicator of soil quality (**Table 1**).

5. Heavy metal contamination diagnosis through change in microbial community structure and diversity

Change in microbial community structure and diversity is a sensitive tool which can be used for diagnosis of heavy metal pollution in soil [47]. Extremely rich microbial diversity in soil [61, 62] can be reduced to 1000 times in a moderately contaminated soil [63] or up to 1% of primitive soils in highly contaminated conditions [64]. Different experiments have been conducted in favor of reduced diversity in metal polluted soils [7, 65] as indicated in the **Table 2**. A reduction in microbial diversity is observed with long term Cr contaminated soil [66]. Microbial community diversity also found decreased with Cu and Zn contamination in long run [68]. Soil remediation techniques show their significance by changing microbial diversity. Use of sepiolite for stabilization of Cr significantly increased community diversity [70]. Iron grit is useful for control of metal contamination (Cd, Cu, Zn) it gives result by improving diversity of microbial communities [71]. Certain findings indicated heavy metal contamination is not always negatively correlated with diversity it may increase diversity [5], while others not found any correlation [62] **Table 2**. Studies indicated that heavy metal contamination directly affects physiology of microbial community thus decreases diversity, Certain communities can withstand this adverse condition while adopting dormant state [62]. Albeit dormancy is an option but it serves the purpose only in short run if exposure is prolong to chronic contamination an obvious adverse effect on functions of community is unavoidable. Communities resistant to contamination may gain their full diversity with time [63]. Soil quality reliably evaluated with Community structure of microbes [42]. Soil microbial community structure significantly changes with heavy metal stress [72, 73]. With long exposure to Cr contamination soil proteobacteria community changed to firmicutes [66]. Pristine soils were dominated with acidobacteria and actinobacteria but population turns into proteobacteria when soil contaminated with Cr, As [62]. Heavy metal contamination may affect one population while not affecting the other one. A study conducted by indicates

Heavy metal	Changes in diversity and structure	Research methodology	References
Cr	Decrease in diversity, community changes	16S r RNA sequencing	[66]
Cu, Zn, Pb	Decrease in diversity, community changes	Pyrosequencing and PFLA techniques	[67]
Cu, Zn	Decrease in diversity, community changes	Metagenomics and functional assays	[68]
Cd	Decrease in diversity, community changes	Metagenomics	[7]
As, Pb	Decrease in diversity, community changes	PCR-DGGE	[69]
Cu	No significant change in diversity, community structure changes	16S r RNA tagcoded pyrosequencing	[63]
V	Diversity first decreases then increases, community structure changes	PCR-DGGE	[5]

Table 2.
Heavy metal pollution with relation to diversity and structure of microbial community in soil.

that Cu contamination changes the community composition for bacteria without affecting fungal community [74]. Heavy metal stress affects bacterial population most than archaea [19]. Archaea shows a positive correlation with Cd while bacterial species exhibit different responses towards Cd like α-proteobacteria shows negative correlation, β-Proteobacteria are positively correlated, γ-proteobacteria and δ-proteobacteria does not show any correlation. Different response of proteobacteria can be explained with complex lifestyle of proteobacteria, it can use different organic matter as a carbon, and energy source [75] this ability enables them withstand in harsh conditions and respond differently to different environments. Different microbial interaction may also help microbes to a better adaption [19]. Consistent conclusion about sensitivity of microbial diversity and structure is not available; one cannot clearly explain which one is more sensitive indicator. Bacterial diversity must be more sensitive than bacterial community structure for heavy metal stress [67]. Soils contaminated with neutral mine effluent and sediments [76] changes bacterial structure significantly than their diversity [77]. It was investigated that both diversity and structure of bacterial population changed under Cd contamination [65]. Increased diversity and structural improvement of microbial community ensures better functioning of soil in heavy metal contaminated soils [74]. In heavy metal contaminated soil sensitive species are replaced with more tolerant species thus it increases species richness [78]. Community dynamics also affected by species evenness [79]. Hence relation between diversity and structure is complex, both need to be use simultaneously in order to evaluate soil quality of a heavy metal contaminated soil. Species richness and evenness may not change simultaneously under stress condition. Mn contamination in soil affects species richness but not evenness to the significant level [80]. In all the previous studies related to heavy metal contamination importance has given to species richness very few literature considered species evenness [80]. Different modern techniques of new era improved our understanding towards cellular constituents like fatty acids, protein, nucleic acid and other compounds related to any specific taxa which proved helpful in recognizing diversity and structure of bacterial community in contaminated soils. Pros and cons of different techniques cannot be avoided; different techniques show certain deviation from other technique **Table 3**. Pyrosequencing does not indicated any significant change in bacterial community structure of a heavy metal Cu, Zn and Pb contaminated soil but using PLFA analysis a significant

Method	Applicability	Advantage	Limitation	References
PLFA	Microbial community	Indicator of living microorganism; act as a biomarker for community structure and physiological state microorganism	Interpretation of PLFA method is difficult; microbial diversity cannot be assessed: Temperature and nutrition can change fatty acid structure; Single acid cannot represent any specific species	[67, 81]
DGGE	Gene cluster; microbial community structure	Sample can be analyze under temporal and spatial variation; easy to operate; multiple samples can be analyzed at a time	It can provide information sequence between primers; if a primer is mismatched it will lead some missing lineages; it only isolates <500 bp fragments effectively; it only detect the microorganism but cannot give any information about species richness.	[82]
ARDRA	Microbial community structure	Identify closely related sequence effectively and inexpensively	Cannot identify polygenetic group; restriction enzyme optimization with this technique is difficult	[83]
High-throughput sequencing	Microbial diversity and community structure	Helpful in tracking biomarker so characteristics of microbial community can be determined	Expansive; data accuracy may get spoiled by some invalid sequence	[84]
T-RFLP	Microbial community	High sensitivity and better resolution	Interpretation needs multiple restriction enzymes; This technique is highly dependent on PCR amplification of 16S/18S r RNA	[81]

PLFA: Phospholipids fatty acids; DGGE: Denaturing gradient gel electrophoresis; T- RFLP: Terminal-restriction fragment length polymorphism; ARDRA: Amplified ribosomal DNA restriction analysis.

Table 3.
Different methods for determination of community structure of microbes.

change is observed [67]. Soil environment also play a significant role in expression of microbial communities in contaminated soils. Soil pH had a significant role in affecting community composition in long term Cu contaminated soil [74]. Soil microbial community structure and diversity not only serves as an indicator of detrition of soil quality but it also predict ways to remediate a deteriorated soil. Metagenomics helps one to understand complicated communities of microorganisms and their working process along with unique ability for identification of new strains and genes [85]. Thermophilic cyanobacterium MTP1 genome is helpful in encoding different resistant system, mainly Cd, Cu, As, Co, Zn, Hg contaminated soils, Which indicates greater potential of this microorganism in remediation of metal contaminated soils [86]. Certain microorganism which are tolerant to contamination for example proteobacteria are tolerant to Cd contamination, possibly can be used to deal with soil Cd contamination [7]. Microbial abundance is less sensitive than microbial community structure and diversity as a indicator for metal contamination [21, 34], but sole dependence on these indicator is not advisable for

determination of soil quality. These two indicators do not reflect functioning of system. Different microbial communities may have similar functions which causes superfluity, and in some cases even though microbial diversity is high but activity may be low [48]. However activity of microbial community may recover in long run but it may change its community structure.

6. Diagnosis based on enzyme activity

Sol enzymes, most important component which governs nutrient cycling in soil specially C, N and P cycle [87]. Enzyme system stability and sensitivity makes it an effective indicator of biochemical processes, Hence enzyme system behaves as a biological indicator helpful in diagnosing sol health [87]. High enzyme activity of soil represents good sol health while in presence of pollutant enzyme activity may reduce [88]. Quantitative relation between soil pollution and enzyme activity is not established till today hence only the change in soil enzyme activity after and before contamination is analyzed for determination of soil quality. Sufficient literature is present to support that enzymes are sensitive towards heavy metal pollution [40, 87]. When a contaminated soil is compared with non-contaminated soil dehydrogenase enzyme activity decreased with heavy metal (Cu Cd Zn Pb) contamination [48]. Vanadium (V) concentration shows negative correlation with urease activity [5]. Response of soil enzymes can vary in different ways to heavy metal contamination it may be activation, inhibition and neutral. Most of the studies indicate the depressed enzyme activity, and inhibition may depend on concentration of heavy metal [45]. The mechanism is not certain whether heavy metal direct inhibit enzyme activity or they reduces their release or both the mechanisms are operative simultaneously [89]. Heavy metal seriously inhibit enzyme activity, but with time some recovery was observed [90]. This may be because of sudden exposure to heavy metal contamination but with time microorganism adapt to environment and recovery is seen in enzyme activity. Different soil enzymes react differently to heavy metal stress, it is important to choose the right enzyme which shows maximum response to heavy metal contamination and react as a suitable indicator in determination of soil quality. Enzymes like catalase, urease and dehydrogenase mostly used as bioindicator.

Catalase helps in decomposition of hydrogen peroxide, reduce heavy metal toxicity (Cu, Zn, Pb, As, Cr, and Cd) to microorganisms [87]. Dehydrogenase takes part in oxydative phosphorylation and used in heavy metal contaminated soils [48]. Urease partakes in N cycle and used in V, Zn, Cu, Pb, Ni and Mn contaminated sites [5, 40]. Amylase, phosphatase and protease were also used as biological indicator for metal contaminated sites. Different enzymes have different levels of sensitivity [91] shows that soil contaminated with different heavy metals follow presented order on the basis of their sensitivity; dehydrogenase found highly sensitive followed by urease followed by alkaline phosphatase and lastly acid phosphatases found least sensitive. As and Cd toxicity did not influence dehydrogenase activity [92]. Heavy metal (Cd, Zn and Pb) contaminated soils sensitivity of urease was found higher than other enzymes like invertase, catalase and alkaline phosphatase [93]. Contamination of heavy metals (Zn, Cu, Cd, As, Cr, Ni, Pb) did not affect urease activity significantly [87]. Previously conducted studies and their results indicated that there were many differences during the applicability of experimental results to the actual environment [87]. Synergistic and antagonist relation among different heavy metals also influence their toxicity for enzyme system. In a study conducted by [57] they concluded that combined effect of Cd and Pb was significantly inhibitorier for enzymes (Dehydrogenase, acid phosphatase and urease) than

Cd or Pb alone as a pollutant in the system. Heavy metals (Pb, Cd, Zn) in combination had strong inhibitory action on enzymes (alkaline phosphatase, catalase, invertase and urease) than any single heavy metal [93]. Some researchers found that Cu as a sole heavy metal in a system inhibit enzymes (alkaline phosphatase, acid Phosphatase, dehydrogenases and urease) more than its presence in combination with Cd, Cr, Pb, Ni and Zn. Type of heavy metal and content in a system determines antagonistic or synergistic relationship of heavy metals. Effect of heavy metal on soil enzymes will also be determined by environment (soil grain size, soil organic matter, pH, etc.). Particle size distribution explains the Zn pollution and enzyme resistance to the pollution [94].

Target enzyme	Participation enzyme	Pollutants	Results	References
Catalase	Dehydrogenase, β-glucosidase, urease, alkaline phosphatase, arylsulphatase	As, Cd, Cr, Cu, Hg, Mn, Pb, Zn	Negative correlation	[95]
	Polyphenoloxidase, catalase, amylase, acid phosphatase, urease	As, Cd, Pb, Zn	Positive correlation, polyphenoloxidase was the most sensitive soil enzyme	[87]
	Catalase, alkaline phosphatase, dehydrogenase	Cd, Pb	Negative correlation	[90]
Dehydrogenase	Alkaline phosphatase, dehydrogenases, urease, acid phosphatase	Cd, Cr, Cu, Ni, Pb, Zn	Sensitivity: dehydrogenases > urease > alkaline phosphatase > acid phosphatase	[91]
	Urease, catalase, acid and neutral phosphatase, sucrase	Cu, Zn, Cd, Pb, Ni, Mn	Negative correlation; Sensitivity: dehydrogenase > urease > catalase > neutral phosphatase > sucrase > acid phosphatase	[40]
	Catalase, alkaline phosphatase	Cd, Pb	Negative correlation; Sensitivity: dehydrogenase > catalase, alkaline phosphatase	[90]
	Invertase, urease, arylsulfatase, catalase, alkaline phosphatase	As, Cd	Insignificant	[92]
	Urease	V	Negative correlation; Sensitivity: dehydrogenase > urease	[5]
Urease	Dehydrogenase, catalase, acid and neutral phosphatase, sucrase	Cu, Zn, Cd, Pb, Ni, Mn	Negative correlation;	[40]
Phosphatase	Phosphatase, urease, β-glucosidase, protease	Cd, Ni	Sensitivity: phosphatase > urease > β-glucosidase > protease	[96]
	Catalase, dehydrogenase	Cd, Pb	Negative correlation	[90]

Table 4.
Heavy metal pollution and soil enzymes.

pH also affects enzyme activity in different ways being low and high it controls enzyme activity sites and their dissociation state as well as enzyme stability [87]. Soil organic matter content positively affects soil enzyme activity. There was a quantitative relationship between soil enzymes and organic matter content at Pb concentration of 500 mg/kg, Arylsulfatase activity found higher with organic matter content of more than 1.05%, activity of enzyme decreased gradually with decrease in organic matter content below 1.05% [92]. Dehydrogenase activity was also related to soil organic matter availability [48]. Labile organic carbon not only act as a food source for microorganism but also serve a binding agent for soil particles and in between space of these complexes soil enzymes are being protected [95]. Till now a uniform standard for selection of indicator enzyme is absent, no enzyme serve the purpose of being an universal indicator for soil quality determination. Heavy metals affect different enzymes differently based on their respective environment. All the enzymes used in diagnosis of soilquality can be divided in two classes one oxidoreductase (polyphenoloxidase, catalase etc.) and other one is hydrolases (amylase, urease, phosphatase, etc.). oxidoreductase are bioindicator enzymes, they take part in detoxification of metal contaminated soils hence more sensitive for heavy metal pollution as an indicator [87]. While hydrolases are involved in nutrient cycling hence can be used as auxiliary enzymes. Highly heterogeneous nature of soils demands further verification of this hypothesis over a long time to validate the results. Moreover we need better quantitative relation to understand the nature of heavy metals and enzymes along with their environmental condition (**Table 4**).

7. Conclusion

Different microbiological indices including microbial abundance, diversity structure and function of microbial community have been used to diagnosis of soil health. So far there is not any single method is alone found a suitable indicator of heavy metal pollution. Every indicator has their shortcomings as microbial abundance does not consider population structure change. Community structure does not reflect functions of population. For a better understanding of soil health all the indicators need to be used simultaneously. More study is needed in the direction of heavy metal contamination diagnosis with functional microorganism. Quantitative relationship between physicochemical factors and microbial indicators need to be established in a better way. Harm due to heavy metal on microorganism depends on the speciation and availability of heavy metal not on metal abundance. Heavy metals may change their toxicity after entering the complex soil system [74]. Long term experiments are needed to find the long term effect of heavy metals short term diagnosis of soil quality is unable to reflect long term soil quality changes.

Author details

Sukirtee Chejara[1*], Paras Kamboj[2], Y.V. Singh[3] and Vikas Tandon[1]

1 Department of Soil Science, CCS Haryana Agricultural University, Hisar, India

2 Department of Agronomy, CCS Haryana Agricultural University, Hisar, India

3 Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, India

*Address all correspondence to: spsukirtee35@gmail.com

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